overproduced the terminal oxidase with and without the cydX gene product. The resulting enzyme was purified by chromatographic steps and the cofactors were spectroscopically characterized. We demonstrated that CydX tightly binds to the CydAB complex and is co-purified. The identity of CydX was determined by mass spectrometry. Additionally, the di-heme active site was only detectable in the variant containing CydX. Thus, CydX is the third subunit of the *E. coli* bd oxidase and is essential for the assembly and stability of the di-heme site [3].

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S9.P10

Characterization of the two cbb3-type cytochrome *c* oxidase isoforms from *Pseudomonas stutzeri* ZoBell

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Cytochrome c oxidases (CcOs) are the terminal enzymes of the respiratory chain and are members of the heme-copper oxidase superfamily (HCO). CcOs catalyze the reduction of molecular O2 to water and couple this exergonic reaction with transmembrane proton translocation. Compared to family A and B CcOs, the cbb3-type CcOs which represent the C-family, feature a distinctly different subunit composition, a reduced proton pumping stoichiometry and higher catalytic activity at low oxygen concentrations [1] [2]. The genome of *Pseudomonas stutzeri* ZoBell contains two independent cbb3-operons, encoding Cbb3-1 (CcoNOP) and Cbb3-2 (CcoNOQP). We generated variants with a focus on ccoQ whose function is unknown. The purified variants and the wildtype Cbb3 were analyzed using UV–vis spectroscopy, BN- and SDS-PAGE, O2 reductase activity (ORA) and immunoblotting with an antibody specific for CcoQ. We found that the deletion of ccoQ has an influence on a b-type heme in the binuclear center, and that both the stability and the ORA are decreased without ccoQ compared to the WT. The O2 affinity (OA) of Cbb3 was spectrophotometrically determined with oxygenated leghemoglobin as an O2 delivery system. The determined Km values for the recombinant Cbb3-1 are similar to previously published data [2]. The Km value of rec. Cbb3-2 is about 2-fold higher than the value of rec. Cbb3-1. In addition, the OA and ORA of different variants introduced into the O2-cavity of rec. Cbb3-1 show significant differences compared to the WT. In the structure of Cbb3, an additional transmembrane α helix was detected but so far not assigned to any protein [3]. We sequenced and identified the polypeptide chain using a customized MALDI-Tandem-MS-based setup and found a putative protein. The amino acid sequence of this protein fits the electron density of the unknown helix and we are currently investigating the functional relevance of this protein.

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S9.P11

Expression of terminal oxidases under nutrient-limited conditions in *Shewanella oneidensis* MR-1

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Shewanella species are facultative anaerobic bacteria renowned for their remarkable respiratory versatility that allows them to use, in addition to O_2, a broad spectrum of compounds as electron acceptors. In the aerobic respiratory chain, terminal oxidases catalyze the last electron transfer step by reducing molecular oxygen to water. The genome of *Shewanella oneidensis* MR-1 encodes for three terminal oxidases: a bd-type quinol oxidase and two heme-copper oxidases, a A-type cytochrome c oxidase (Cox) and a cbb3-type oxidase. In a previous study, we investigate the role of these terminal oxidases under aerobic and microaerobic conditions in rich medium using a biochemical approach [1]. Our results revealed the particularity of the aerobic respiratory pathway in *S. oneidensis* since the cbb3-type oxidase was the predominant oxidase under aerobic conditions while the bd-type and the cbb3-type oxidases were involved in respiration at low-O_2 tensions. Against all expectation, the low-affinity Cox oxidase had no physiological significance in our experimental conditions. Do these data reflect a functional loss of Cox resulting from evolutionary mechanisms as suggested by Zhou et al. [2]? Is Cox expressed under specific conditions like the aa3 oxidase in *Pseudomonas aeruginosa*, maximally expressed under starvation conditions [3]? To address these questions, we investigated the expression pattern of the terminal oxidases under nutrient-limited conditions and different dissolved O_2 tensions by measuring oxidase activities coupled to mass-spectrometry analysis. In addition to the notable modulation of the expression of the bd-type and cbb3-type oxidases in the different tested conditions, we detected Cox oxidase under carbon-starvation conditions. This constitutes the first report of a condition under which the A-type oxidase is expressed in *S. oneidensis*. We suggest that Cox may be crucial for energy conservation in carbon-limited environments and we propose that Cox may be a component of a general protective response against oxidative stress allowing *S. oneidensis* to thrive under highly aerobic habitats.

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S9.P12

Determination of H^+/e ratios in mitochondrial yeast cytochrome *c* oxidase

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Cytochrome *c* oxidase (C*c*O) is the terminal enzyme of the human respiratory chain. It reduces the oxygen we breathe into water and conserves the free energy of the reaction in the essential proton gradient that drives ATP synthesis by the ATP synthase. The oxygen chemistry reaction of C*c*O is fairly well understood but the way it is coupled to the energy-requiring proton transfers remains to be elucidated [1]. Two hydrophilic pathways have been identified from the available crystal structures as potential routes for pumped protons, namely the D- and the H-pathway. Mutagenesis studies on chimeric mammalian forms of the enzyme have suggested that the H-pathway is the pathway for pumped protons in bovine mitochondrial C*c*O whereas mutagenesis studies of bacterial C*c*Os indicate that only the D pathway fulfils this role. We have developed yeast *Saccharomyces cerevisiae* as a model system to investigate the pumping mechanism of an additional mitochondrial C*c*O. As both its nuclear and mitochondrial genomes are amenable by mutagenesis, mutations can be made in any part of the C*c*O structure to assess its function [2]. Mutations in both the D- and the H-pathway of the yeast enzyme lead to respiratory growth deficiency [3]. We will present H^+/e ratio measurements based on ADP/O ratios of intact, coupled mitochondria prepared from a series of mutants of the D- and the H-pathway. The results will be discussed in terms of pumping mechanism and of function of hydrophilic channels in all forms of oxidases.

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S9.P13

Comparative pH and temperature dependent studies on different types of terminal oxidases by protein film voltammetry

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Terminal oxidases couple the reduction of oxygen to the translocation of protons across the membrane. This proton translocation is driven by electron transfer to the catalytic site which is a binuclear center composed of a heme and a copper center. As the properties of enzymes can vary depending on the environment where they grow, organisms can express different types of terminal oxidases which can exhibit different pH dependence as well as different thermostability [1]. This family of proteins can also be divided into three subfamilies named A, B and C depending on their first electron acceptor, their number of proton pathways or their relative oxygen affinity [2]. Protein film voltammetry is a powerful technique to study electrochemical and catalytic properties of redox enzymes [3]. However, it is difficult to immobilize a large amount of membrane proteins on an electrode surface. To overcome this problem, we proposed to use a three-dimensional gold nanoparticle network that is known to mediate the long-range electron transfer between the cofactors and the electrode [4]. We report here on the temperature- and pH- dependent electrocatalytic properties of different types of terminal oxidases. Complementary studies by infrared spectroscopy allow us to discuss about the differences observed in the secondary structures at different temperatures and to link them to the previously observed electrocatalytic properties. Clear differences can be observed between the different subfamilies of oxidases.

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S9.P14

Simulation of charge and exciton transfer kinetics in proteins

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Capturing sunlight and converting it to chemical energy is a central process in nature. The complexity and the number of proteins taking